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THE INTERNAL SECRETIONS IN GROWTH AND DEVELOPMENT OF AMPHIBIANS

EDUARD UHLENHUTH, PH.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, N. Y.)

WHILE up to 1910 the higher vertebrates were used predominantly in the study of the internal secretions, during the last decade the larvæ of the amphibians have been found an excellent material, suitable for the investigation of many problems of endocrinology. To-day the results obtained in this work seem to form a solid mass of trustworthy evidence, from which may be derived not only valuable information as to the mechanism of growth and development in amphibians, but also important knowledge as to the functions of certain endocrine glands. In the field of internal secretion, these experiments have attracted increasing interest from the beginning. It is evident at present that further clarification of many of the more important problems of internal secretion will come from the work on amphibians, as it can be and has been carried on with methods far superior to those available in the work on higher vertebrates.

Before entering into details the most prominent facts as revealed in the amphibian experiments may be pointed out. In the control of growth and development of the amphibian organism, the thyroid and pituitary glands play the most important rôles. The thymus is not concerned with the growth and development of amphibian larvæ. The functions of the thyroid and hypophysis glands, as far as they are revealed in the processes of

growth and development, exhibit a remarkable resemblance, and the secretions of these two glands can replace each other to some degree, but for the most part are specific.

Among those who have worked out these facts, J. F. Gudernatsch, Leo Adler, Bennet M. Allen and his pupil W. W. Swingle, E. R. Hoskins and M. M. Hoskins, and P. E. Smith deserve the greatest credit. Since much of their success is due to the extirpation of the endocrine glands in early embryonic stages, we should mention here also the names of three investigators, namely Gustav Born, Herman Braus and Ross G. Harrison, who have elaborated the delicate technique employed in the extirpation experiments and thus have made possible the progress which has been derived from them.

We will begin with the thyroid mechanism, as it has been studied more thoroughly than other glands, and in fact seems to be the chief factor in the control of growth and development. Its study in the amphibians as well as the entire work on amphibians was initiated by the well-known experiments on thyroid-feeding to tadpoles as carried out by Gudernatsch (1).

If tadpoles are fed fresh thyroid gland or are kept in water to which minute amounts of thyroid extract are added, a remarkable acceleration of development takes place. This development is the more conspicuous as it may occur with complete absence of growth. In tadpoles it is characterized especially by the sudden development of the fore limbs, by the atrophy of the tail, a sudden protrusion of the eye-balls (2), by the rapid shortening of the spiral gut, by the precocious atrophy of the organs of the larval mouth, which are replaced by the frog mouth (3), and by precocious ossification (4). These experiments have been repeated with the larvæ of salamanders, in which the precocious occurrences of the first moult, the atrophy of the gills and absorption of the fin of the tail, are most conspicuous effects of the thyroid application (5).

The rapidity with which these processes may take place is one of the most remarkable features of the thyroid effect. Two normal larvæ of the species *A. opacum,* for instance, metamorphosed at an age of 86 days and measured 60 mm. at this time. Six other larvæ of the same brood were placed in an emulsion of iodothyrine at an age of 35 days, at which time they measured 30 mm. on the average. One week later, at an age of only 42 to 43 days and a size of 24 mm., all had metamorphosed. Moreover, 5 days after metamorphosis, *i.e.,* at an age of 47 days in one animal which was examined in sections, the visceral skeleton had undergone all those complicated changes through which the gill arches of the larvæ develop into the hyoid apparatus of the adult. The effect of the thyroid hormone is quantitative; the acceleration of the amphibian metamorphosis increases with increasing concentration of the thyroid emulsion, as shown in Table I.

TABLE I

QUANTITATIVE EFFECT OF THE THYROID HORMONE IN THE ACCELERATION OF THE METAMORPHOSIS OF *Ambystoma maculatum*

Quantity of Iodothyrine	Age at Metamorphosis		
	Control	Iodothyrine	Difference
0.1 gm. iodothyrine in 1,000 c.c. of water	101 days	33 days	67 per cent.
0.01 gm. iodothyrine in 1,000 c.c. of water	80 "	58 "	28 " "

In one experiment the larvæ of *Ambystoma maculatum* were kept in water, to which 0.1 gm. of iodothyrine per 1,000 c.c. of water had been added; in another experiment only 0.01 gm. of iodothyrine was added to 1,000 c.c. of water. In the first experiment all larvæ metamorphosed 13 days after the first application of iodothyrine; in the second experiment metamorphosis took place 39 days (on the average) after the first application of iodothyrine. The difference between the normal time of metamorphosis and the time of metamorphosis of the experimental larvæ was 67 per cent. in the first experiment and 28 per cent. in the second.

It is remarkable that the administration of the same amount of iodothyrine causes metamorphosis of salamander larvæ of different species in nearly the same interval of time. Thus, 0.1 gm. iodothyrine per 1,000 c.c. of water caused metamorphosis of *A. opacum* larvæ in 7 days, of *A. maculatum* larvæ in 13 days and of *A. tigrinum* larvæ in 13 days. The time required to induce metamorphosis in thyroid-fed tadpoles decreases with increasing age of the tadpoles. Gudernatsch (1) found that thyroid feeding caused metamorphosis in 20 days if tadpoles of a certain age were employed, in 6 days, if tadpoles 7 days older than the first lot were employed, and in only 4 days if the tadpoles were 14 days older than the first lot.

As pointed out above, the larvæ, when fed thyroid substance, may undergo the most remarkable development, although no growth may take place. This seems to be of great importance in many ways. In all organisms development and growth, under normal conditions, proceed in a parallel way. The behavior of the thyroid-fed larvæ suggests that the reason why no development takes place without growth is the fact that, under normal circumstances, the substances which cause development of certain organs are supplied through the same reactions which control the growth of the organism. If these substances are supplied to the organism from without, development may proceed at a higher rate than growth or may proceed even in the complete absence of growth and thus the relation between growth and development may become changed as in the thyroid-fed larvæ.

The changes of the relation between growth and development furnish an important link in the chain of facts that we must know in order to understand the mechanism of the thyroid apparatus as well as that of the amphibian metamorphosis. Although under certain conditions growth may be inhibited completely upon the feeding of thyroid, this is not always the case. Both the rate of development and the rate of growth are dependent

on the quantity of thyroid substance administered to the larvæ. Up to a certain quantity, growth as well as development is accelerated; if the quantity administered is further increased, growth becomes more and more inhibited, while differentiation is increasingly accelerated. With very large doses the thyroid substance may effect even a decrease in the size and weight of the larvæ; while development of the limbs is greatly accelerated in the beginning, it finally stops and the animals die from emaciation (6).

Kendall (7) has shown that in man the thyroid hormone increases the basal metabolism in a strictly quantitative way. Determinations of metabolism have not been made in amphibians, but the behavior of the thyroid-fed tadpoles as described above indicates that the thyroid hormone also increases highly the metabolism of the cold-blooded organism. If too much of the hormone is administred, metabolism is increased in such a manner that catabolism becomes higher than anabolism, since the organism no longer is capable of supplying enough food materials from outside to maintain a positive metabolic balance, and consequently the body substance itself is broken down and a decrease in size and body weight takes place. Finally even development becomes impossible. For this reason, as Lenhart (6) showed, more thyroid substance can be administered without leading to a check of development if the thyroid-fed larvæ are either kept under conditions which decrease metabolism, *i.e.,* in low temperature, or are fed on substances (carbohydrates) which can be made easily available for metabolism.

From these facts it seems evident that the amphibian metamorphosis is the result of a highly increased metabolism, or more correctly, metamorphosis seems to result if metabolism is increased in such a degree and manner that catabolism becomes higher than anabolism. The question arises whether substances or agents other than

thyroid substance can cause such an increase of metabolism as to bring about metamorphosis. Several experiments have been carried out to answer this question. But until thyroidectomized tadpoles have been employed in these experiments, no definite conclusions are possible; in larvæ possessing a normal thyroid gland it can not be decided whether the experimental conditions employed have caused metamorphosis by raising the metabolism directly or merely through the intermediation of the thyroid by precociously releasing the thyroid hormone. Powers as well as Barfurth has shown that a sudden cessation of food supply results in precocious metamorphosis. Although this is certainly true, it does not decide the point in question, but may mean that sudden starvation may precociously release the thyroid hormone. At any rate, starvation in itself does not cause metamorphosis, but is effective only if well-fed larvæ which are approaching metamorphosis and possess a thyroid capable already of functioning are suddenly starved. The same criticism applies to Kaufman's (8) recent experiments, in which an advanced neotenous larva (axolotl) of *Ambystoma tigrinum* was given salicylic acid, whereupon it metamorphosed promptly. This result is extremely interesting as it raises most urgently the question whether the action of iodothyrine is specific and whether the changes of metabolism resulting from thyroid administration are merely quantitative or also qualitative. As pointed out, Kaufman's experiment, however, does not answer any of these questions.

In accord with the highly increased catabolism as caused by the action of the thyroid hormone is the fact that metamorphosis, in its initial stages, appears to be more a process of profound atrophy than one of constructive development, although phenomena of the latter kind frequently accompany the degenerative processes. Among the most conspicuous processes of destruction are the complete atrophy of the gills and the entire vascular apparatus which serves the gill circulation, a con-

siderable destruction of the cartilaginous visceral skeleton, the atrophy of the larval mouth in anurans, the reduction of the intestinal coils in anurans, the complete atrophy of the tail in anurans and the atrophy of the fin in urodelans. Not before this extensive breakdown of the larval tissues has taken place and out of the remnants of the destroyed organs the new organs of the adult develop. Particularly instructive in this regard is the development of the epithelial bodies in the larvæ of salamanders; these develop from the epithelium of the destroyed gills and in the midst of the masses of detritus which result from the destruction especially of the gill vessels.

The fact that metamorphosis can be brought about by feeding mammalian thyroid substance to the amphibian larvæ, does not of course prove that the amphibian metamorphosis, under normal circumstances, is the result of the function of the amphibian thyroid gland itself. This, however, is the case, as demonstrated especially by the work of Allen (9) and of E. R. and M. M. Hoskins (10). If in an early embryonic stage of the anuran organism the thyroid is extirpated, metamorphosis can not take place at all and the tadpoles remain permanently (as far as the observations go) in the stage of an aquatic amphibian larva. Growth likewise is ultimately interfered with, although the thyroidectomized tadpoles may grow more rapidly in the beginning and even grow larger than normal tadpoles. On the other hand, if the thyroid of metamorphic tadpoles is grafted to tadpoles which are in early larval stages, metamorphosis of the latter, up to the stage of the larvæ from which the thyroid graft was taken, is caused (11). The metamorphosis of the amphibian eye is likewise impossible if it is removed from the influence of the thyroid hormone which controls the development of the eye. If eyes of old salamander larvæ are grafted to young larvæ, the metamorphosis of the graft may be retarded by as many as 7 months and will

not take place before the eyes of the host metamorphose. On the other hand, eyes of young larvæ, if they are grafted to old larvæ, can be made to metamorphose earlier than they would under normal conditions (12).

It has been said that the thyroid substance does not actually *produce* new characters, but merely accelerates the rate of their development which is predetermined by heredity. There can be little doubt, however, that the advance of the higher vertebrates from an aquatic stage, with open gill slits and internal or external gills, and in particular all the characters distinguishing the terrestrial amphibian from the aquatic larva, could not have developed if the thyroid apparatus had not attained, at some evolutionary stage of the amphibians, its present function. For the benefit of those who might think that the relatively short time (about $1\frac{1}{2}$ years) of observation in Allen's and Hoskins's experiments does not justify this statement, I may refer to the Texan cave salamander, *Typhlomolge rathbuni* which illustrates in a most vivid manner the effect of the absence of the thyroid gland. This salamander never develops beyond the larval stage, retaining permanently its external gills and other larval organs. An examination of the endocrine system of this animal was made by Emerson (13); it revealed the complete absence of the thyroid gland. It is worth while to mention briefly another interesting condition observed in this animal, namely the almost complete lack of pigment, a condition somewhat similar to that observed by Smith and by Allen in hypophysectomized tadpoles, and the highly atrophied state of the eyes. *Typhlomolge* is a white, blind salamander. These latter peculiarities have been attributed frequently to the absence of light in the caves, a theory which at first seems very plausible. It would not be surprising, however, if some day these characters should be found to be the result of endocrine disturbances. Similar to *Typhlomolge* in all the characteristics mentioned above is a European salamander, *Proteus anguineus,* which inhabits the Austrian lime-

stone caves; nothing, however, is known about the endocrine glands of this animal.

If the thyroid substance is capable of causing the development of the characters of a terrestrial amphibian, the administration of thyroid substance should cause metamorphosis of *Proteus anguineus*. Jensen (14) subjected *Proteus* to the action of thyroid substance, but did not get any demonstrable results. Many causes may have been responsible for this failure, in particular the fact that the animals were too old when they were subjected to the thyroid feeding.

It has been known for some time that the effect of equal doses of thyroid substance on the amphibian metamorphosis is the greater, the more iodine there is contained in the thyroid gland (15). Recently, Swingle (16) has demonstrated that the feeding of common inorganic iodine to tadpoles or the keeping of the tadpoles in iodine solutions accelerates metamorphosis in the same way as does the thyroid. This effect of iodine is strictly quantitative; if there is no iodine contained in the food of the tadpoles, metamorphosis is inhibited, while with an increasing amount of iodine metamorphosis is increasingly accelerated. Moreover, the effect on the relation between growth and development is the same in iodine solutions and in thyroid feeding. Weak solutions of iodine increase not only the rate of development, but also the rate of growth, while high concentrations prevent growth. There can be no doubt that at least in the metamorphosis of tadpoles, iodine is an indispensable constituent of the thyroid hormone.

Swingle (16) found that potassium iodide and iodoform had an effect on metamorphosis similar to that of iodine, while bromine had no effect on metamorphosis and growth. Thus the effect of iodine appears to be very specific when comparison is made with so nearly related a substance as bromine.

The feeding of iodine to mammalians does not produce the same effects as the administration of thyroid sub-

stance. This fact has formed the basis for the opinion (7) that the characteristic action of the thyroid hormone is not directly caused by the presence of iodine in the thyroid hormone. It seems, however, more probable that the feeding of iodine has no effect on mammalians, because the mammalian organism, for some reasons, can not utilize an excess of iodine. It is well known that the mammalian thyroid gland is capable of storing large amounts of iodine (17). If, under normal conditions, only a definite amount of hormone could be excreted by the thyroid gland, the feeding of excess amounts of iodine would have no effect in the healthy individual, since every excess of iodine would be retained and stored by the thyroid tissue. If in the mammalian organism the thyroid gland should be the only organ capable of elaborating the thyroid hormone, the feeding of iodine could have no effect in the absence of the thyroid, or in persons whose thyroid function is insufficient. Conditions are different with tadpoles. Swingle (16) has shown that even in thyroidectomized tadpoles, iodine solutions are capable of causing metamorphosis. Apparently the thyroid gland is not the only organ of the tadpole which can produce the thyroid hormone.

It should be pointed out, however, that a fundamental difference exists between frogs and toads on the one hand, and salamanders on the other, as regards their reaction to iodine. Salamanders behave much like mammalians. Although I was able to confirm the accelerating action of iodine at least in the development of the limbs of the tadpoles, I have not been able to cause precocious metamorphosis by placing salamander larvæ in iodine solutions. Table II will illustrate this statement.

Two larvæ of the species *A. maculatum* were kept first in a solution of 5 drops $\frac{1}{20}$ m. iodine per 1,000 c.c. water and then, up to metamorphosis, in a 3-drops-iodine solution. No acceleration of metamorphosis took place; the larvæ metamorphosed at an age of 122 days, while the controls were only 101 days old when they metamor-

phosed. It is interesting to note that while tadpoles of *Rana sylvatica* are killed by a 5-drops-iodine solution and upon a 3-drops-iodine solution respond promptly with development of the hind limbs, the larvæ of *A. maculatum* showed no other effect from a 5-drops solution than a slightly decreased food intake. The latter circumstance may account for the longer duration of the larval period of the experimental larvæ. Since it was believed that in this experiment the solution was too weak, 2 larvæ of the same species, after a short sojourn in a 3-drops solution, were placed in an 8-drops-iodine solution; but as Table II shows, in this experiment also metamorphosis was not accelerated by the iodine solution. Several larvæ were fed directly crystals of iodine to make sure that the ineffectiveness of the iodine solution in salamanders was not due to a possible impermeability of the larval skin for iodine. In one case two crystal-fed larvæ metamorphosed at 124 days, while the controls metamorphosed at the age of 101 days. In another experiment, in which 3 larvæ were employed, one metamorphosed at the age of 89 days, while the controls metamorphosed at 80 days. Of the two other larvæ, one did not show any signs of metamorphosis when it was killed for histological examination; the other one died from an overdose of iodine, but did not show any sign of metamorphosis.

TABLE II

IODINE HAS NO EFFECT ON THE METAMORPHOSIS OF *A. maculatum*

Quantity of Iodine.	Age at Metamorphosis.		
	Normal.	Iodine Solution.	Iodine Sol. + Crystals.
5 to 3 drops 1/20 m. iodine in 1,000 c.c. water	101 days	122 days	124 days
5 to 8 drops 1/20 m. iodine in 1,000 c.c. water	80 "	79 "	89 "

Three old larvæ, all of the axolotl type, and one neotenous, of the western race of *Ambystoma tigrinum*, which were collected in the Rocky Mountain lakes last

summer, were subjected to an iodine treatment. They were placed in water containing 5 drops of a ½0 m. solution of iodine per 1,000 c.c. of water and, as they showed no reaction of any kind, this concentration was increased gradually to 8 drops and in one larva to even 13 drops of iodine (3 drops of a ½0 m. solution of iodine per 1,000 c.c. of water is enough to cause growth of the hind limbs in larvæ of *Rana sylvatica*), which is more than 0.2 c.c. of a ½0 m. solution of iodine per 1,000 c.c. of water. Although these larvæ have now been in the iodine solution for 2 months, none of them has developed any tendency towards metamorphosis, while 3 other control larvæ, among them a neotenous specimen, metamorphosed 13 days after being placed in an emulsion of 0.1 gm. of Bayer's iodothyrine per 1,000 c.c. of water. Evidently the assumption suggested by Swingle (16, III), that lack of iodine prevailing in the lakes is causing the inhibition of metamorphosis of the axolotl and other urodelans, is unwarranted. I will show presently that in the inhibition of metamorphosis and in neoteny of axolotls and probably certain European urodelans we are confronted with an entirely new phase of internal secretion, namely with the differential action of temperature upon the development of various components of the endocrine system.

In a former article (20) I suggested that the inhibition of metamorphosis in thymus-fed amphibian larvæ may be caused by lack of iodine in the thymus. Swingle (16, III) has accepted and unfortunately repeated, without further testing, this suggestion. But recent experiments show that this view must be abandoned, since addition of iodine to a pure thymus diet does not enable the salamander larvæ either to grow or to metamorphose. Similarly the retardation of growth and metamorphosis of salamander larvæ kept on a pure diet of posterior lobe of hypophysis remains unaffected if iodine is added to the water.

The iodine requirement of salamanders must be extremely slight, since anterior lobe of hypophysis, a nearly

iodine-free diet, does not in any way retard growth or metamorphosis.

There are several species of salamanders (*Autodax lugubris, Autodax iecanus*) whose young do not emerge from the eggs before metamorphosis is completed. Although the larvæ of these species have no opportunity to obtain iodine from outside, these cases do not prove, of course, anything against the importance of iodine in the amphibian metamorphosis; very likely the eggs of *Autodax* contain enough iodine to permit metamorphosis of the larvæ within the egg.

Still another difference between anurans and salamanders has made itself apparent in this work. While in tadpoles, of at least certain anuran species, the development of the legs is, in some as yet unknown way, distinctly under the control of the thyroid, the leg-development in salamanders is independent of the thyroid gland. Both hind and fore limbs develop in a normal way after thyroidectomy in salamander larvæ, as shown by E. R. and M. M. Hoskins (10). Moreover, the development of the legs is not accelerated if the larvæ are kept in solutions of iodothyrine (18); this is the case even if the administration of iodothyrine is commenced soon after the eggs have been deposited. Consequently, it is very common to find that the larvæ metamorphose in the iodothyrine solution before the legs are completely developed. It is evident that in tadpoles part of the larval development is controlled by the thyroid function, since neither the hind limbs, from a certain stage on, nor the fore limbs can develop in the absence of the thyroid (9, 10, 19). Apparently the anuran thyroid gland begins to secrete already in the larval period. In salamanders the larval development seems to be highly independent of the thyroid function and it is quite probable that the salamander thyroid does not begin to function much before the first moult. This can be demonstrated in the following way (12). If eyes of old larvæ which, however, are still far enough from metamorphosis, are grafted on to young

larvæ, their metamorphosis is inhibited until the host metamorphoses. If the eye graft, however, is taken from larvæ which are near metamorphosis, such an inhibition is no longer possible. Apparently shortly before metamorphosis actually occurs, the thyroid begins to excrete, and after the circulating hormone has reached the eye metamorphosis of the eye takes place, even if the organ is transferred to an animal in which the thyroid hormone has not yet been secreted.

It is quite possible, that the late beginning of the thyroid function in salamander larvæ is one of the causes why the administration of an excess of iodine is ineffective in the metamorphosis of these amphibians. Probably the thyroid merely stores up the excess of iodine, but does not release the hormone till shortly before the first moult.

Allen (19) has recently examined the condition of the thyroid of Colorado axolotls and has found that they possess a thyroid corresponding in size, structure and colloid content to the thyroid of adult specimens of *A. tigrinum*. The thyroid of the larvæ of other salamander species likewise seems to be mature much before metamorphosis actually takes place. Allen concluded from his observations that the thyroid of salamanders begins to function at an early stage of the larvæ. The independence of the larval development of the salamander larvæ as demonstrated by the facts mentioned above shows, however, that the presence of a mature thyroid before metamorphosis must be interpreted in a different way. The most conspicuous character in the salamander metamorphosis is the fact that, although it certainly is dependent on the thyroid hormone, it does not necessarily take place in larvæ whose thyroid is mature. This can only mean that two factors are required in order to bring about the metamorphosis of salamander larvæ, namely a mature thyroid gland and a factor which releases the thyroid hormone from the follicles of the gland.

This conception, which is now supported by several

facts, is also capable of explaining the problem of neoteny of the so-called axolotl. In the course of experiments carried on during several years in the laboratory, and by inspection of the conditions prevailing in the Rocky Mountain lakes, the natural habitat of the American axolotl, I have become convinced that the neoteny of this species is due to the effect of low temperatures. We have in the amphibians an experimental material in which the relation between the development of the body and certain endocrine glands can be changed by the influence of temperature, owing to the differences of the temperature coefficients of the processes governing the development of different glands.

Although my experiments are not yet finished, they seem to permit the following conclusions in connection with my field observations:

1. The thyroid gland of salamanders undergoes a developmental change consisting of two periods, one of early development, lasting at least 63 weeks, in the course of which the thyroid becomes more and more sensitive to the action of a releasing factor (called excretor substance in my earlier work) and one of aging in the course of which the thyroid loses gradually its sensibility to the releasing factor.

2. In order to release the hormone of the thyroid gland, a particular releasing factor is required (the nature of which is entirely unknown); the quantity of this factor necessary to release the thyroid hormone depends on the sensitivity of the thyroid gland. Metamorphosis can take place only if the thyroid is sensitive and is acted upon by the proper quantity of the releasing factor.

3. The temperature coefficient for the elaboration of the releasing factor is higher than the temperature coefficients for growth and the thyroid change.

The following facts seem to warrant these assumptions:

1. Salamander larvæ, kept at an identical temperature, are nearly all of the same size when they metamorphose. Larvæ kept at low temperatures grow considerably larger

than those kept at high temperature, before they can metamorphose. This is shown in Table III (2). The temperature coefficient for the releasing factor is higher than that for growth.

TABLE III

TEMPERATURE AND SIZE OF THE METAMORPHOSING LARVÆ

Species	Series	Size in Mm.		Series
		25° C.	15 C.°	
Opacum	A 1916	57	67	C 1916
	XIV 1918	61	71	XVIII 1918
Tigrinum	S 1917	102	119	U 1917
	XLVI 1919	103	122	XLVIII 1919
Maculatum	LXXV 1920	52	59	LXXVII 1920

2. In very low temperatures (6° C. to 10° C.) growth is greatly slowed down and consequently the elaboration of the releasing factor must be still more retarded; yet larvæ kept at 6° C. grow less and less large before metamorphosis, when they are transferred, at increasing ages, to 15° C., as shown by an experiment lasting 63 weeks thus far. Apparently the thyroid has gone on to mature at a relatively high rate and at 63 weeks is highly sensitive and responds to smaller quantities of the releasing factor. The temperature coefficient for the thyroid change is considerably lower than those for growth and for the elaboration of the releasing factor.

3. If the thyroid can continue to develop in the absence of growth, it probably can also commence to age. Should this assumption be correct, the larvæ kept at 6° C. should finally become unable to metamorphose, if the time during which they are kept in 6° C. is sufficiently long. At present this assumption would explain why many specimens of the Colorado axolotl yield only slowly, if at all, to the influence of high temperature, and the Mexican axolotl frequently loses completely its ability to metamorphose.

4. The Colorado axolotls reach frequently a size considerably in excess of the normal maximum size of the

species as calculated from the largest known terrestrial specimens of the eastern race of this species; the Colorado axolotls are giants. Since sexually mature specimens of the eastern race of *A. tigrinum* become giants if they are fed anterior lobe of hypophysis, the gigantism of the sexually mature axolotl could be explained if any indications of hyperpituitarism of these animals could be discovered. On the assumption that in spite of the presence of a large thyroid the function of this organ is suppressed by the absence of the releasing factor, the overfunction of the axolotl hypophysis would be very plausible, since, as will be pointed out later on, the absence of the thyroid function causes hypertrophy of the hypophysis in amphibian larvæ.

5. The maturing of the sex organs of the axolotl is not incompatible with the assumption of an athyroidism, since, as will be discussed presently, there can be no longer any doubt that the development of the sex organs of amphibians is entirely independent of the thyroid hormone.

6. The assumption that the temperature effect can actually produce the complex phenomenon of neoteny is supported by the fact that the species *A. tigrinum* becomes neotenous only in the high and cold regions of the Rocky Mountains and the Mexican high plateau, while in the eastern part of the United States all individuals of this species metamorphose in a normal manner. I have examined the conditions prevailing in the Rocky Mountains; to summarize briefly my observations, the axolotl is regularly found only in those lakes which are permanently exposed to low temperatures, while in the shallow lakes of lower altitudes axolotls are found only during some years and are absent during other years; apparently a succession of several years favorable in temperature conditions is required to produce the axolotl state.

7. *A. tigrinum* is the only species of North American salamanders which becomes neotenous. This is probably

not due to differences existing between the endocrine system of the numerous species inhabiting the United States, but is explained by the fact that *A. tigrinum,* among the closely related species which I had an opportunity to test, is the only species that can stand temperatures low enough to bring about the necessary difference between the rate of the thyroid development and that of the elaboration of the releasing factor.

8. The fact that many individuals among the offspring of female specimens of the Mexican axolotl do not metamorphose even if they are brought, immediately after hatching, into conditions permitting normal metamorphosis of other salamander species, is not necessarily related to the factors discussed above, but may be due to the development of congenital thyroid disturbance in the young born by an athyroidous female.

It is, of course, well known that many structural changes, only a few of which have been studied, are required to make, out of the aquatic larvæ, the terrestrial amphibian. This is true for the anurans as well as for the urodelans. Since we know that the complex phenomenon of metamorphosis is initiated by the thyroid effect, the question arises now which of the component changes are directly caused by the action of the thyroid hormone. The fact that certain developmental processes frequently take place upon thyroid administration and therefore are a very convenient indicator in studying quantitatively the effect of thyroid substance, of iodine or of any other metamorphosis-causing agent, does not mean, in itself, that these developmental processes are caused directly by the action of the thyroid; it is possible and indeed supported by many facts, that certain of these changes will follow automatically, after the initial changes have been effected by the thyroid action. Thus, while under normal conditions, the pigmentary pattern, the legs, the tongue, the palatal teeth and the sex organs mature in salamanders during metamorphosis, they can be shown to be highly independent of

the thyroid action at least in salamanders and may, under certain conditions, occur in the absence of this action or not occur in the presence of it. I have pointed out in former articles (18) that among the many changes occurring during the salamander metamorphosis there are two which seem to be particularly closely related to the thyroid action, namely the first shedding of the skin and the reduction of the gills to mere stubs. While the succession of all the other changes enumerated above seems extremely variable, the order in which the first moult and the reduction of the gills follow each other could not be changed as yet by any of the procedures employed, inasmuch as the shedding of the skin always is followed by the atrophy of the gills. Moreover, these two phenomena have never failed to occur in the metamorphosis of the many hundreds of metamorphosing larvæ observed in the laboratory, and even in such larvæ as were forced at a very early date into precocious metamorphosis by the administration of iodothyrine and in which other changes did not occur. And furthermore, neither the first moult nor the reduction of the gills could ever be observed in larvæ, whose metamorphosis was inhibited by dietary or other means. Thus I have come to look upon the first moult and the atrophy of the gills as two of the primary components of the salamander metamorphosis. I have not enough personal experience with the larvæ of anurans, but feel encouraged through the experiences reported by other investigators to believe that in anurans these phenomena play a similarly important rôle. Certainly the first shedding of the skin seems to accompany true metamorphosis in salamanders and tadpoles as well (38), and substances other than thyroid hormone or iodine, such as the anterior lobe substance, although they may cause the limbs to grow, do not bring about atrophy of the gills in thyroidectomized tadpoles (27).

It is different with the limbs, the pigmentary pattern, the tongue, the palatal teeth and the sex organs; these five groups of organs, at least in salamanders, have

proved to be little influenced by the thyroid action. That the development of the limbs of salamanders is not dependent on the thyroid gland has been pointed out above; here I may add that Typhlomolge is a further illustration of this fact, as in this salamander the legs develop in a normal manner in spite of the complete absence of the thyroid gland. The relation of limb development and thyroid action in tadpoles is by no means definitely settled as yet. In tadpoles the development of the limbs seems to be highly dependent on the action of the thyroid gland; but attention has been called to this surprising difference between two groups of organisms so closely related otherwise and the suggestion has been made in a previous article (18), that this difference as far as the fore legs are concerned may be due merely to the fact that in tadpoles the limbs grow beneath the skin and consequently can not break through unless the changes are initiated which finally lead to the shedding of the skin and that these changes and not the thyroid action are the primary factor in the development of the anuran fore limbs. Whether or not this assumption is correct can not be decided at present, but certainly deserves renewed attention in view of recent discoveries which demonstrate that the development of the limbs of tadpoles, at least in certain species, is not as dependent on the thyroid secretion as some investigators were inclined to think. Allen (34), who has made prolonged observations in thyroidectomized tadpoles, has recently found that not only the hind limbs, but even the fore limbs in the thyroidectomized larvæ of *Bufo* ultimately attain a size and differentiation not only equal but superior to those attained in normal metamorphosing larvæ. The only difference, however, is that in the absence of the thyroid gland the fore limbs can not break through the skin.

As to the skin pigmentation, it is well known that larvæ in which metamorphosis has been inhibited for some reasons may develop a nearly adult pigment pattern. In larvæ of *A. opacum* which were fed thymus gland, and

consequently did not metamorphose, the coloration of the skin advanced to a stage very similar to that of an adult animal. On the other hand if young larvæ of *A. opacum* are made to metamorphose precociously by means of the application of iodothyrine, metamorphosis takes place, while the color pattern remains in an early larval stage. Through the observations of Cope (35) it has become known that otherwise completely metamorphosed individuals of the species *A. tigrinum* may exhibit either a larval condition of the tongue or larval characters of the palatal teeth or larval characters in both the tongue and the palatal teeth.

In nature it is not uncommon that the sex glands of salamanders develop to complete maturity while the rest of the organism remains in a larval stage (18). This phenomenon, known by the name of neoteny, illustrates that the sex organs can develop in the absence of the thyroid function. The same fact has been shown in the larvæ of anurans by B. M. Allen and his coworkers. In thyroid-fed frog larvæ, which have undergone precocious metamorphosis, the sex organs do not seem to be further developed than those of normal larvæ of the same age (3). Moreover, if the thyroid is removed from the larvæ and metamorphosis inhibited, the sex organs develop at the same rate as in normal larvæ (21). Hoskins (22) and Allen (21) showed that the testicle of thyroidectomized tadpoles may develop ripe spermatozoa. These facts, however, can not be interpreted to mean that the germ plasm is independent of the somatic plasm, in the Weismannian sense. The characteristic feature of the amphibian development is not the independence of the germ plasm from the somatic plasm, but the independence of various groups of organs from one another, due to the fact that the development of each of these groups is controlled by substances different from those controlling the other groups, and that each of these substances separately may be supplied to or withheld from the organism either by the experimenter or by conditions

not fully known as yet (18). One of these conditions is the temperature as has been pointed out above.

I will discuss briefly now the rôle of the hypophysis in the growth and development of amphibians. The most noteworthy fact seems to be the existence of a remarkable resemblance between the functions of the amphibian thyroid and hypophysis glands during the larval period. If the hypophysis gland is extirpated in early embryonic stages, the tadpoles stop to develop at a stage at which, in normal tadpoles, metamorphosis begins. Growth, too, is inhibited in the hypophysectomized tadpoles (23, 24). In a series of extremely interesting experiments Allen (25) showed that both growth and development can be restored to the hypophysectomized tadpoles, if the anterior lobe of the hypophysis of an adult frog is grafted to such larvæ. No other part of the hypophysis when grafted to the hypophysectomized tadpoles can restore growth and development, and it is certain, therefore, that it is the anterior lobe of the hypophysis which controls the growth and development of the larvæ. In tadpoles the feeding experiments as made by P. E. Smith (26) seem to corroborate the extirpation experiments. Feeding of anterior lobe to hypophysectomized tadpoles increases the rate of growth to such an extent that growth becomes as vigorous as in normal larvæ. Moreover, at the time when the normal tadpoles metamorphose and growth ceases for a time, the anterior lobe-fed hypophysectomized tadpoles continue to grow and finally attain a size in excess of that of normal larvæ. Ultimately, however, the growth of these larvæ stops and before the size is reached characteristic of the normal adult animal. The effect of feeding anterior lobe to normal larvæ is a matter still under discussion at present. Smith (26) found that normal tadpoles when fed anterior lobe grew apparently at a slightly higher rate and also metamorphosed at a slightly earlier date than normally fed tadpoles. Recently, however, Smith (36), on account of the considerable variation in the rate of growth and develop-

ment of normal larvæ, seems to be inclined to consider these differences as being of no significance. Certainly it is of no small importance that normal and hypophysectomized larvæ react so differently to a diet of anterior lobe substance; apparently part of the active principle of the anterior lobe introduced, by the diet, into the organism is made ineffective in the presence of a normal hypophysis. Not yet completed experiments on salamander larvæ seem to suggest that the larval growth of salamanders at least can not be affected by feeding anterior lobe of hypophysis; this may be due either to a destruction of the active principle in the digestive tract or to some peculiarity in the metabolism of the salamander larvæ, and is of particular interest with regard to the fact that the adult salamanders react very markedly to an anterior lobe diet, as will be discussed presently.

One of the most pertinent and yet most difficult problems of endocrinology is presented by the existence of interrelations and interactions between the various endocrine glands. There can be no doubt that in tadpoles such an interrelation exists between the hypophysis and thyroid glands. Thus Rogers (31) and later on Hoskins and Hoskins (22) found that upon thyroidectomy performed in early embryonic stages of anurans the anterior lobe of the hypophysis shows a tendency towards hypertrophy. On the other hand if the buccal anlage of the hypophysis is removed, the thyroid soon ceases to grow and to differentiate and finally presents a state of hypoplasia, as shown by Allen (30) and by Smith (36). Since the effects of the extirpation of either of these glands on general body growth and on development are quite similar and since the behavior of each of these glands after the extirpation of the other one demonstrates the existence of an interrelation between them, the question might well be asked, if the function of each of these glands can not be replaced by the hormone of the other one of them. Although this question can not be satisfactorily answered thus far, it seems highly probable that these hormones

are strictly specific in as much as neither of them can replace the function of the missing one. To quoting the inhibition of metamorphosis and growth following hypophysectomy as proof in favor of this view one could object that in this particular case the thyroid can not effect metamorphosis and growth merely on account of its atrophic condition. Smith (36), however, found, that in certain cases of partial hypophysectomy the thyroid remains completely unaffected and yet no metamorphosis takes place; only if the remaining fragment of the epithelial hypophysis grows large enough to come in contact with the neural hypophysis, metamorphosis can be effected. For this reason Smith takes the view that the function of the hypophysis is indispensable in metamorphosis and that the secretion necessary for this purpose can only be elaborated, if epithelial and neural hypophysis are in contact with each other. That neither the anterior nor the posterior lobe of the hypophysis contains the substance necessary for metamorphosis and that this substance can be produced only in the body itself, requiring for its elaboration the contact between neural and buccal hypophysis, seems much supported by the fact that, although growth may be maintained up to a certain size, by feeding anterior lobe to hypophysectomized tadpoles, metamorphosis can not be effected in such tadpoles by feeding either anterior or posterior lobe. As to the possibility of replacing the function of the anterior lobe substance by introducing into the organism thyroid hormone or iodine, Allen (28) fed iodine to hypophysectomized tadpoles and obtained some, but not all of the changes induced by iodine in normal and thyroidectomized larvæ and seemed to be tardily inclined to the view that the lack of the hypophsis could be compensated for by feeding iodine. Smith, however, in his last publication (36), claims that neither thyroxin nor thyroid gland itself causes metamorphosis, when fed to pituitaryless tadpoles. Quite similar are the results of feeding hypophysis to thyroidectomized larvæ. Hoskins and Hoskins (27) were able to cause growth of limbs and emaciation

by feeding anterior lobe substance to thyroidectomized tadpoles, but could not obtain complete metamorphosis; especially the atrophy of the tail and of the gills could not be enforced. Similarly Allen (37) points out that feeding anterior lobe of cattle does not result in metamorphosis of thyroidectomized tadpoles.

If taken together, all these results seem to indicate that although certain resemblances exist between the hormones of the thyroid and the hypophysis glands, they are nevertheless specific and can not replace each other as regards at least certain functions.

As pointed out above, the metamorphosed salamanders react on anterior lobe feeding quite differently from the larvæ. Such differences in the reaction upon the same principle in different stages have been observed quite frequently and are apt to throw an important light on the nature of the chemical reactions involved in growth and development of different stages. The salamander larvæ show no appreciable effect from an anterior lobe diet, whether the anterior lobe be fed alone or in small quantities added to normal food. If metamorphosed salamanders of the species *A. opacum* or *A. tigrinum* are fed anterior lobe, the rate of growth becomes almost immediately accelerated and growth continues after the animals have reached the specific maximum size of the species; they become giants. The latter result must be attributed to the action of a specific growth promoting hormone contained in the anterior lobe (32).

The thymus gland apparently has no effect on growth and development, although it has been believed that it contains specific growth-promoting and development-retarding substances. It is true that in larvæ which are fed on thymus only, growth as well as metamorphosis are inhibited. The inhibition of metamorphosis, however, is due to the fact that in the absence of growth the releasing factor of the thyroid can not form, as has been mentioned above. Moreover, the inhibition of growth is not caused by specific hormones of the thymus, but is merely a deficiency phenomenon. The more normal food

there is added to the thymus, the less marked does the inhibition of growth become; small amounts of thymus added to a normal diet have no effect (33). It is unknown at present which of the food substances necessary for growth are missing, although it is certain that the deficiency of the thymus is not caused by a deficiency in iodine, calcium, sodium or potassium. Many other glands, such as the spleen, prescapular lymph-gland, parathyroids, and posterior lobe of the hypophysis are more or less deficient in the growth of salamander larvæ.

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